Claims 1 and 17 are rejected under 35 U.S.C. §102(e) as being anticipated by U.S. Patent 6,331,166 issued to Nakamura. Claims 1 and 17 are rejected too under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent 5,925,534 issued to Miki and by Sugiuchi (Clin. Chem.) Claims 2-4, 5-9, 12, 14-15 and 18-27 are also rejected under 35 U.S.C. § 103(a) as being obvious over Sugiuchi.[1/]

Regarding the rejection for anticipation, the claims are amended to recite that a reagent enabling the CH enzymes to act only on LDL cholesterol is a combination of a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer. This subject matter at least is not taught by the prior art. Accordingly, the rejection for anticipation is overcome.

Claims 2-9, 12, 14-15 and 18-27 are rejected under 35 U.S.C. 103 as being unpatentable over Sugiuchi in view of U.S. Patent 5,773,304 issued to Hino et al.

Sugiuchi teach a selective method for measuring LDL cholesterol in serum without the need of prior separation, using the non-ionic surfactant polyoxyethylene-polyoxypropylene copolymer (hereinafter referred to as "POE-POP") and α-cyclodextrin sulfate, which is a sodium salt of sulfated cyclic maltohexaose. Sugiuchi teaches that a combination of POE-POP with α-cyclodextrin sulfate provided the required selectivity for the determination of LDL cholesterol in serum. That is, of Sugiuchi requires α-cyclodextrin sulfate for the determination of LDL cholesterol.

Hino is directed to a method for determining cholesterol in high density lipoprotein using a surfactant and a substance which forms a complex with lipoproteins other than HDL. The surfactant used in Hino's method includes a polyoxyethylene derivative and POE-POP.

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1/ As understand, the basis for the rejection over Sugiuchi is 35 U.S.C. §102(b).

On the other hand, the present invention is broadly directed to the determination of LDL cholesterol using a combination of a polyoxyethylene derivative and a POE-POP.

The present invention and Sugiuchi both relate to the determination of LDL cholesterol. However, a polyoxyethylene derivative and a POE-POP are used for selectively determining LDL cholesterol in the present invention, while a POE-POP and α-cyclodextrin sulfate are used for the same purpose in Sugiuchi. Therefore, the present invention is clearly distinguished from Sugiuchi. Moreover, this deficiency in the primary reference is not overcome by Hino.

Rather, Hino is directed towards determining HDL cholesterol, which plainly differs in kind from that of the present invention. Hino is completely silent about determining LDL cholesterol.

Further, a salient feature of the present invention is its utilization of a combination of a polyoxyethylene derivative and a POE-POP to provide an excellent selectivity for the determination of LDL cholesterol by inhibiting the interaction between enzymes and cholesterol in lipoproteins other than LDL.

In contrast, Hino is directed to a method for determining HDL cholesterol which comprises contacting a sample containing HDL and lipoproteins other than HDL with a surfactant and a reagent, forming a complex of the reagent with the lipoproteins other than HDL; and determining the amount of HDL cholesterol. Hino teaches that such surfactants inhibit the interaction between the enzymes used for the detection of cholesterol and lipoproteins. (e.g., cholesterol in lipoproteins other than HDL).

In this regard, the Examiner states (see section 7 of the Office Action) that Sugiuchi discloses a method for the measurement of LDL cholesterol using non-ionic surfactant and POE-POP. Respectfully submitted, this statement is based on a misunderstanding of Sagiuchi. As described above, Sugiuchi uses POE-POP and α-

cyclodextrin sulfate, which is an ionic surfactant, POE-POP being the only non-ionic surfactant used in Sugiuchi. Further, the Examiner states

> Sugiuchi et al. teach that · · · the reactivity of cholesterol in VLDL and chylomicron may be suppressed by a non-ionic surfactant; Sugiuchi et al. specifically exemplifies α-cyclodextrin sulfate.

See from page 5, last line to page 6, line 3 of the Office Action.

However, again, α-cyclodextrin sulfate is an ionic surfactant. For instance, note the following description at Sugiuchi at page 522, lines 1 to 6, left column

> We have developed a fully automated method for measuring LDL cholesterol · · · using a non-ionic surfactant, polyoxyethylene-polyoxypropylene block copolyether (POE-POP), and a sodium salt of sulfated cyclic maltohexaose, α-cyclodextrin sulfate.

For that matter, note the following description at Sugiuchi at page 526, line 6 from the bottom et seq, left column

> Our previous studies have shown that α-cyclodextrin sulfate ····· reduces the reactivity of cholesterol, especially in chylomicrons and VLDL in the presence of magnesium ions ·····.

Thus, Sugiuchi makes clear that the reactivity of chylomicrons and VLDL are suppressed not by a non-ionic surfactant, but by α-cyclodextrin sulfate.

In any event, the Examiner further states as follows:

> It would have been obvious to one having ordinary skill in the art at the time of the invention to have specifically measured LDL cholesterol by inhibiting the reactivity of cholesterol in other lipoproteins using a POE-POP, as specifically taught by Sugiuchi et al., and to have replaced the non-ionic surfactant, α-cyclodextrin sulfate, with another appropriate non-ionic surfactant such as a polyoxyethylene derivative as taught by Hino et al.

The Examiner's assertion about the replacement of the non-ionic surfactant has no bases in fact. Since α-cyclodextrin sulfate is not a non-ionic surfactant, it cannot be replaced with Hino's polyoxyethylene derivative. Further, as is described above, the

purpose of the polyoxyethylene derivative in Hino differs in kind from that in the present invention.

The Examiner writes:

> "A motivation for utilizing a polyoxyethylene derivative in combination with the polymer taught by Sugiuchi et al. is provided by Hino et a1. who teaches that polyoxyethylene derivative inhibits the interaction between enzymes and lipoprotein cholesterol and that polyoxyethylene derivatives, including POE-POP condensation products and polyoxyethylene alkyl ethers, may be used in combination."

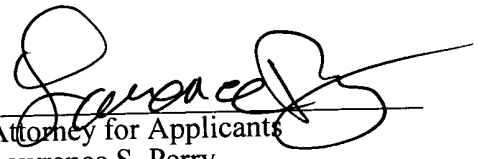
As noted above, Hino teaches that polyoxyethylene derivatives inhibit the interaction between enzymes and cholesterol in lipoproteins other than HDL. In a method for determining cholesterol in LDL using enzymes, there is no reasonable expectation of success from using a polyoxyethylene derivative that inhibits the interaction between the enzymes and cholesterol in lipoprotein other than HDL taught by Hino because it is understood that the polyoxyethylene derivative also inhibits the interaction between the enzymes and cholesterol in LDL. So, there is no reason to utilize Hino's polyoxyethylene derivative with Sugiuchi. In addition, while Hino states that the combination of polyoxyethylene derivative and a POE-POP may be used for quantitatively determining cholesterol in HDL, there is no data showing the effect of the combination, and Hino explicitly teaches the combination may have a selectivity for the determination of HDL and not LDL. Thus, the prior art explicitly teaches away from the present invention.

In view of the above amendments and remarks, Applicants submit that all of the Examiner's concerns are now overcome and the claims are now in allowable condition. Accordingly, reconsideration and allowance of this application is earnestly solicited.

Claims 2-6, 8-9, 12-15, 18-20, 22-24 and 27-33 remain presented for continued prosecution.

Applicants' undersigned attorney may be reached in our New York office by telephone at (212) 218-2100. All correspondence should continue to be directed to our below listed address.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE TO CLAIMS

1. (Cancelled)

2. (Amended) A [The] method [according to claim (1), wherein the reagent enabling] for quantitatively determining LDL cholesterol in a biological sample, which comprises:

(I) reacting cholesterol in the presence of:

a) a biological sample,

b) CH enzymes selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol esterase and cholesterol dehydrogenase, and

c) a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable the CH enzymes to act only on LDL cholesterol [is a reagent containing at least a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer] to form hydrogen peroxide or reduced coenzyme; and

(II) measuring the amount of the hydrogen peroxide or reduced coenzyme.

4. (Amended) The method according to claim 2 [or 3], wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by [general] formula (I):

$$HO—(C_2H_4O)_a—(C_3H_6O)_b—(C_2H_4O)_c—H \quad (I)$$

[()]wherein a, b and c[, which may be the same or different, each] independently represent[s] an integer of 1 to 200[)].

5. (Amended) A method for [the] continuous fractional determination of HDL cholesterol and LDL cholesterol in a biological sample, which comprises:

(I) subjecting cholesterol to [the] reaction in the presence of:

a) a biological sample,

b) CH enzymes selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol esterase and cholesterol dehydrogenase, and

c) a reagent enabling the CH enzyme [of b)] to act only on HDL cholesterol to form hydrogen peroxide or reduced coenzyme, [and]

(II) measuring the amount of the hydrogen peroxide or reduced coenzyme [formed by the first reaction] to quantitatively determine the concentration of HDL cholesterol, then adding [d)] a [reagent enabling] polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable the CH enzymes [of b)] to act only on LDL cholesterol[,];

(III) subjecting cholesterol to the [second] reaction to form hydrogen peroxide or reduced coenzyme[,and];

(IV) measuring the amount of the hydrogen peroxide or reduced coenzyme [formed by the second reaction] to quantitatively determine the concentration of LDL cholesterol.

6. (Amended) A method for [the] continuous fractional determination of HDL cholesterol and LDL cholesterol in a biological sample, which comprises:

(I) conducing a first reaction of [subjecting] cholesterol [to the first reaction] in the presence of:

a) a biological sample,

b) CH enzymes selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol esterase and cholesterol dehydrogenase, and

c) a reagent enabling the CH enzymes [of b)] to act only on HDL cholesterol to form hydrogen peroxide or reduced coenzyme, and

(II) measuring the amount of the hydrogen peroxide or reduced coenzyme [formed by the first reaction] to quantitatively determine the concentration of HDL cholesterol, then adding [d)] CH enzymes, and [e)] a [reagent enabling] polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable the CH enzymes [of d)] to act only on LDL cholesterol,

(III) conducting a second reaction of [subjecting] cholesterol [to the second reaction] to form hydrogen peroxide or reduced coenzyme, and measuring the amount of the hydrogen peroxide or reduced coenzyme [formed by the second reaction] to quantitatively determine the concentration of LDL cholesterol.

7. (Cancelled)

8. (Amended) The method according to claim [(7)] 5 or 6, wherein the polyoxyethylene derivative is a polyoxyethylene alkyl ether or a polyoxyethylene alkylaryl ether.

9. (Amended) The method according to claim [(7)] 5 or 6, wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by [general] formula (I):

$$HO—(C_2H_4O)_a—(C_3H_6O)_b—(C_2H_4O)_c—H \quad (I)$$

[(]wherein a, b and c[, which may be the same or different, each], independently represent[s] an integer of 1 to 200[)].

10. (Cancelled)

11. (Cancelled)

12. (Amended) The method according to [any one of] claim[s] (5) or (6), wherein the reagent enabling CH enzyme to act only on HDL cholesterol [in HDL] is a reagent for aggregating lipoproteins other than HDL.

14. (Twice Amended) The method according to claim (12), wherein the reagent for aggregating lipoproteins other than HDL is a reagent comprising a divalent metal salt and at least one member selected from the group consisting of heparin or a salt thereof, phosphotungstic acid or a salt thereof, dextran sulfuric acid or a salt thereof, polyethylene glycol, sulfated cyclodextrin or a salt thereof, and sulfated oligosaccharide or a salt thereof [or a mixture thereof and a divalent metal salt].

15. (Twice Amended) The method according to claim (6), wherein the CH enzymes used in the first reaction [of cholesterol] are chemically modified enzymes

and the CH enzymes used in the second reaction [of cholesterol] are enzymes that are not chemically modified.

16. (Cancelled)

17. (Cancelled)

18. (Amended) A [The] reagent for determining LDL cholesterol [according to claim (17), wherein the reagent enabling the] comprising CH enzymes [is a reagent containing at least] selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol esterase and cholesterol dehydrogenase, and a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable the CH enzymes to act only on LDL cholesterol.

20. (Amended) The reagent according to claim (18) [or (19)], wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by [general] formula (I):

$$HO—(C_2H_4O)_a—(C_3H_6O)_b—(C_2H_4O)_c—H \quad (I)$$

[(]wherein a, b and c[, which may be the same or different, each] independently represent[s] an integer of 1 to 200[)].

21. (Cancelled)

22. (Amended) A [The] reagent kit [according to claim (21), wherein the] for continuous fractional determination of HDL cholesterol and LDL cholesterol comprising a first reagent [enabling] comprising CH enzymes selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol esterase and cholesterol dehydrogenase, and [to act only on LDL cholesterol is a] a reagent [containing] for aggregating lipoproteins other than HDL, and a second reagent comprising a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable CH enzymes to act only on LDL cholesterol.

23. (Amended) The reagent kit according to claim [(21)] 22, wherein the [reagent enabling CH enzyme to act only on LDL cholesterol is a reagent containing a] polyoxyethylene derivative [and] is a polyoxyethylene[-polyoxypropylene copolymer] alkylaryl ether.

24. The reagent kit according to claim [(21) or] (22), wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by [general] formula (I):

$$HO—(C_2H_4O)_a—(C_3H_6O)_b—(C_2H_4O)_c—H \quad (I)$$

[(]wherein a, b and c[, which may be the same or different, each] independently represent[s] an integer of 1 to 200[)].

25. (Cancelled)

26. (Cancelled)

27. (Three Times Amended) The reagent kit according to [any one of] claim[s (21), (] 22[) or (23)], wherein the reagent for aggregating lipoprotein other than HDL [lipoprotein] is a reagent comprising a divalent metal salt and at least one member selected from the group consisting of heparin or a salt thereof, phosphotungstic acid or a salt thereof, dextran sulfuric acid or a salt thereof, polyethylene glycol, sulfated cyclodextrin or a salt thereof, and sulfated oligosaccharide or a salt thereof[, or a mixture thereof and a divalent metal salt].